GERMINATION- AND GROWTH-INHIBITING SUBSTANCES IN RICE GRAINS II. EXAMINATION OF SINGLE FRACTIONS OF INHIBITORS FOUND IN THE COVERING STRUCTURES

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Introduction

In a previous paper (VARGA, 1964) the effect and major properties of water-soluble germination- and growth-inhibiting substances in rice grains were studied. According to the results these inhibitors obtained from the rice-bran inhibit, dependent on the concentration, the germination of rice embryos and other seeds. Further, they decrease the growth of the root and shoot of the seedlings. The water-extract of the bran can reduce, to a considerable extent, the effect of IAA and GA on the cell elongation and on the stimulation of the germination, respectively. This inhibitory effect can be completely reversed only by a relatively larger quantity of IAA or GA.

In this second part of the investigations we aimed at the separation of some fractions of different solubility from the water extract of rice bran and the examination of their effect and properties.

Material and method

To carry out the examinations Dunghan Shali variety of 1964. was employed. The bran contains the tissues of the husk, pericarp and testa and besides the aleuron layer of the endosperm.

The inhibiting substances were extracted as described in the earlier publication (VARGA, 1964), from 100 g of bran with 200 ml of water. The proteins were precipitated with acetone and after removing the sedimented proteins and the acetone the inhibitory materials of different solubility were fractionated as published by MIYAMOTO et al. (1961). (Table I.). After the evaporation of the organic solvents the residues were taken up in equal quantity of water.

The germination- and growth-inhibitory effect and properties of these fractions were measured with rice-embryo and lettuce seed test, rice and oat coleoptile section test, further with lettuce hypocotyl test, in the same way as in the first part.

Results and discussion

The extract obtained from the crude water extract following the removal of proteins and acetone contains all the water-soluble inhibitors found in the covering structures of rice grains. The germination- and growth-inhibitory effect of this

extract was considered as 100% and the effectiveness of the fractions separated from, was related to this „total activity". The aim of the fractionation according to the solubility was to determine the different types of inhibitors and to obtain data concerning their chemical nature.

Examination of germination- and growth-inhibiting effect of the different fractions. Fig. 1 shows the effect of the different fractions exerted on the germination of rice embryos and lettuce seeds. On the basis of these data it can be concluded that about 55 to 60% of the „total activity" (1) is represented by the chloroform-insoluble fraction (2) while 40 to 45% by the chloroform-soluble fraction (3). About 60% of the inhibitory effect of chloroform-insoluble fraction is caused by the ether-soluble acidic fraction (5) and 40% by the ether-insoluble

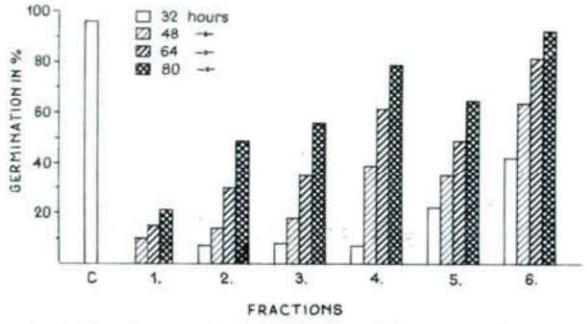

Fig. 1. Effect of the single fractions on the germination of lettuce seeds.
1=water extract of total activity, 2=chloroform-insoluble fraction, 3=chloroform-soluble fraction, 4=ether-insoluble fraction, 5=ether-soluble acidic fraction, 6=ether-soluble neutral fraction.

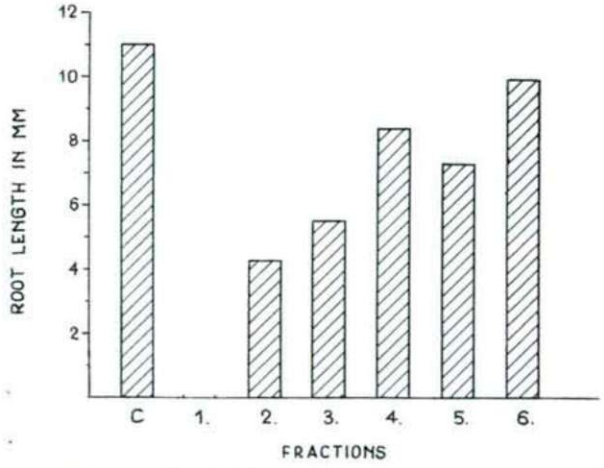

Fig. 2. Effect of the single fractions on the root growth.
C=control, 1—6. as in Fig. 1.

water fraction (4). However, it was proved that a part – about 30% – of the substances not soluble in ether at pH 3 is transformed into ether in alkaline conditions (6).

This different inhibitory effect of the fractions is well manifested not only in the germinating power and per cent but also in the root growth of the seedlings (Fig. 2). Measuring the root length the same conclusion may be drawn as mentioned above.

Behaviour of the fractions to heat. During the previous examinations (VARGA, 1965) was noted that the water extract of rice bran – after 48-hour treatment – lost its activity, proportionately to the rise of temperature, 10 to 15% in the rice embryo test and 11 to 17% in the lettuce seed test. On the other hand, treatment of 100°C increased the inhibitory effect with 5 to 8%. The latter phenomenon was attributed to the fact that other, more active inhibitory substances were produced due to the formation of certain decomposition products, i. e. to the reaction of some components. Now the question arises, which fractions and to what extent are responsible for the activity-changes caused by heat treatment.

The water solution of the substances in the fractions separated from the rice bran extract were kept for 24 and 48 hours at 60 and 100° C, respectively. Thereafter their effectiveness was again determined with lettuce seed test (Fig. 3). The results indicate that the chloroform-insoluble fraction (2) consists mainly of ther-

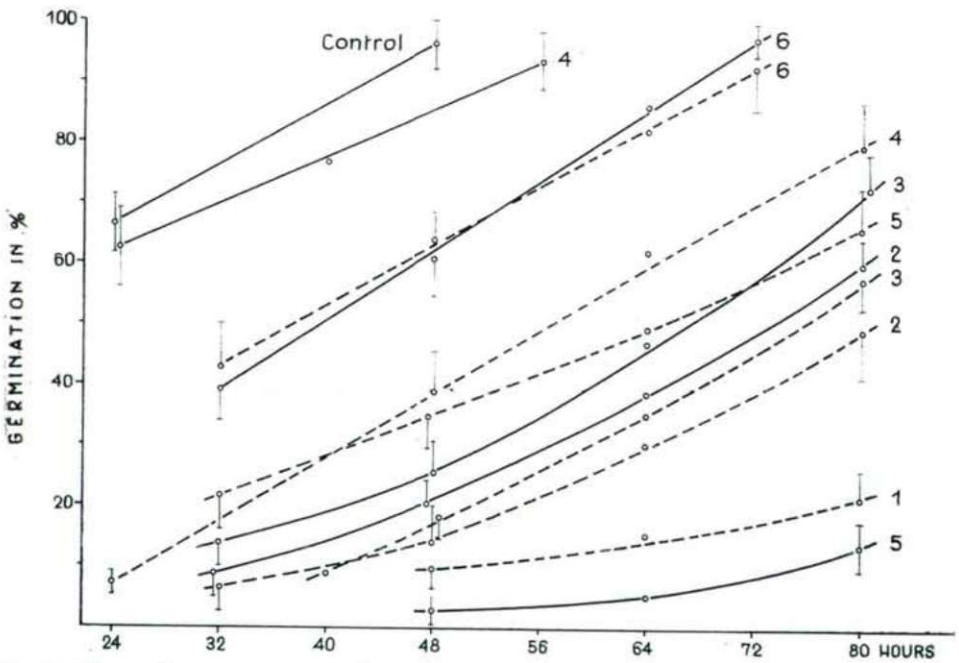

Fig. 3. Effect of heat treatment on the germination-inhibitory action in lettuce seed test.
1—6. as in Fig. 1.
dropped line=effect before heat teatment
continuous line=effect after heat treatment

mostabile components, namely, following heat treatment its germination inhibiting effect only insignificantly (5 to 8%) changed. Consequently, the components in the chloroform-soluble fraction may chiefly be responsible for the activity-changes. The inhibitory activity of the ether-soluble acidic part of this fraction (5) was considerably – about 30 to 50% – increased after 100°C treatment, thus, the aforesaid changes, increasing the effectiveness, assumably occurred in this fraction. The ether-insoluble fraction (4) contains strongly thermostabile inhibitors: these almost completely lost their activity following heat treatment. Whereas, the ether-soluble neutral- fraction (6) was proved to be thermostabile.

Thus, the activity decrease due to the heat treatment experienced in the water extract of „total activity" was produced by the ether insoluble (4) fraction and the

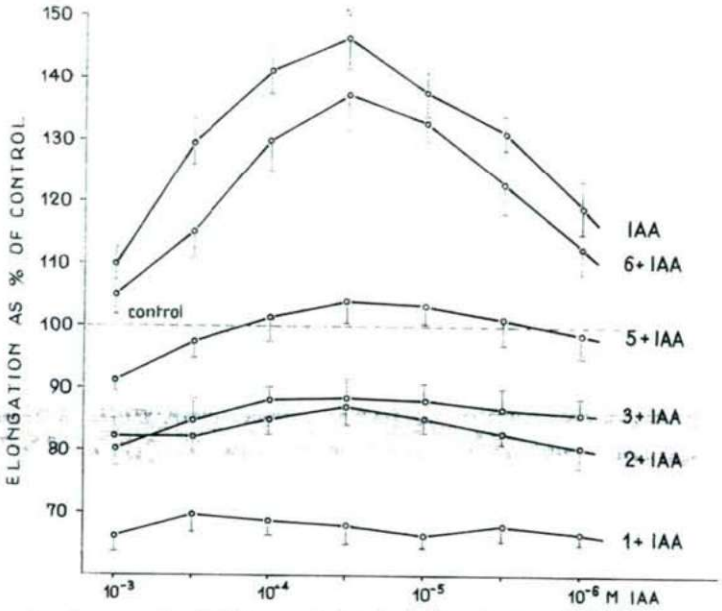

Fig. 4. Interaction between the inhibitors of the single fractions and IAA in oat coleoptile section test.
1—6. as in Fig. 1.
($s=\pm 4{,}2$; $s_x=\pm 0{,}63$; $s\%=5{,}02$; $n=50$)

activity increase by the components of the ether-soluble acidic fraction (5). In the total extract (and in the chloroform-soluble part) naturally the resultant of the changes observed separately in the single fraction, is manifested.

Interaction between the single fractions and IAA. In the first part of the investigations it was shown that the bran extract, dependent on the concentration, reduces the stimulatory effect of IAA on cell elongation in the rice and oat coleoptile section test. In the present paper the effect of the single fractions was examined from this view-point. The results show (Fig. 4) that in this reduction of

the action of IAA, the chloroform-insoluble and the chloroform-soluble fractions take part almost at the same rate.

Those of the chloroform-soluble inhibitors, soluble in ether at acidic conditions, still considerably reduce the cell elongation due to IAA, however, in this case the IAA at 5.10^{-5} to 10^{-5}M concentrations is able to reverse to a remarkable extent the inhibitory activity. The ether-soluble neutral inhibitors alone, result a fairly slight growth reduction.

Interaction between the single fractions and GA. The single fractions of rice bran extract reduce the germination and growth stimulatory effect of GA in similar proportion to that mentioned above. Fig. 5 shows the results obtained in the lettuce hypocotyl test. The response of hypocotyls to GA was best reduced by the chloroform-insoluble inhibitors, however, the effect of the chloroform-soluble part is roughly the same too. Within the latter fraction, the effect of ether-soluble acidic inhibitors are predominant, whereas, the components in the ether-soluble neutral fraction are almost without any effect.

It is quite obvious from the diagrams that the growth inhibitory activity of the single fractions can be gradually reversed by rising the GA concentration. In the case of the least effective fractions 4 and 6, GA in 10 and 100 ppm concentrations entirely nullify the inhibitory action.

From the observations recorded in this study the presence of several germination- and growth-inhibiting substances of different types can be stated. These. separated according to their solubility cause a well noticeable inhibition, even individually, in seed germination and cell elongation. However, together mutually intensify their individual effect; thus, the total activity is higher than the sum of the effect of the single fractions. Such synergism manifested among the phenolic growth inhibitors has been treated in an earlier paper (VARGA, 1957).

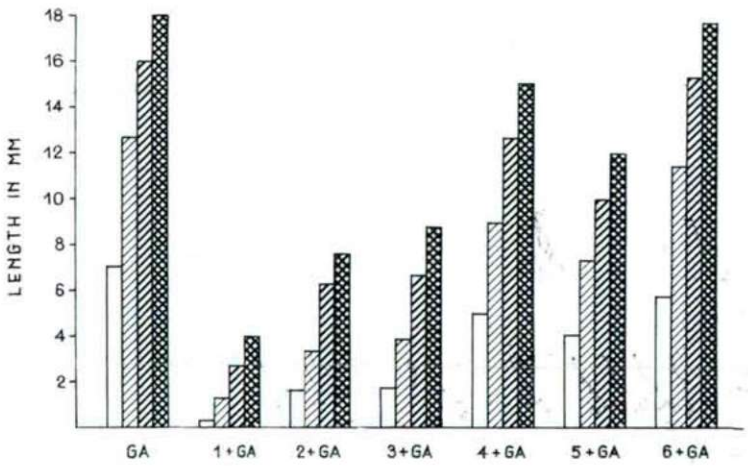

Fig. 5. Interrelationships in the effect of inhibitors of single fractions and GA in lettuce hypocotyl test.
1—6. as in Fig. 1.
($s = \pm 1{,}84$; $s_{\bar{x}} = \pm 0{,}33$; $s\% = 13{,}3$; $n = 75$)

Despite the chemical identification of the inhibitors in rice grains is under way and will be the subject of a next study, certain statements may be already made. The effect and behaviour of the single fractions, non the less the treatment of the chromatograms with common chemical reagents, indicate that the chloroform-insoluble fraction consists mainly of tannic acids, whereas the chloroform-soluble fraction of phenolic acids (5) and other of non-acidic phenolic compounds (6). In addition to the phenolic compounds inhibitors of other type also occur in the extract. As to the predominant phenolic compounds may be assumed that they resemble greatly to the germination and growth inhibitors demonstrated in oat grains and husk, cereal straw, as well as in the pericarp of different dry fruits (KÖVES 1958, KÖVES and VARGA 1958, 1959, BÖRNER 1955, 1956a, 1959b,) including the inhibitors determined in rice grains by MIKKELSEN and SINAH (1961).

Summary

From the water extract of rice grains 5 different fractions were separated on the basis of their solubility in organic solvents as well as on the basis of their reaction to heat treatment. The effect of the single fractions on the seed germination and on the growth of the seedlings, was studied. About 55 to 60% of the total activity can be attributed to the chloroform-insoluble and 40 to 45% to the chloroform-soluble fraction. Within the latter, the inhibitory effect is partly due to the ether-soluble acidic substances and partly to the ether-insoluble components. The single fractions play role, roughly in the same degree, in the activity of the total extract reducing the growth stimulation caused by IAA and GA. Concerning the chemical nature of the inhibitors tentative efforts have been made too.

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Table I. Fractionation procedure for inhibitors in rice bran